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Effects of Cry Protein Based Diet on the Intestinal Motility and Histopathological Changes in Male Albino Rats

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Abstract

In vitro assessment procedure for gastrointestinal motility through Dale's examination of small intestine, following 3 months treatment with Bt Cry protein, shows more avoidance in digestive development in regarded creature when contrasted with control. T3 test gathering of treated rodents is more conspicuous among them. Various examples of digestive developments are engaged with the physiological movement of chyme along the gastrointestinal tract (GIT) and are the consequence of the exchange between unconstrained action of digestive smooth muscle, intestinal and outward neural circuits. The investigation of gastrointestinal motility might be useful in deciding modification in motility, assessing impact of neurotic condition on GI travel and helps in deciding the restorative capability of medications in motility disorders. Expanded motility because of Bt Cry protein meddles with the assimilation and retention measure, in this manner influencing the gastrointestinal motility which can upset usefulness of the GIT, as parasympathetic incitement development though nerves hinder peristaltic developments, which can prompt the runs and the malabsorption condition. Histological sectioning of specified tissue such as small intestine, large intestine and stomach was routinely stained with haematoxylin-eosin (H/E) staining and observed microscopically. Bt Cry protein does not affect the stomach tissue or does not cause any damage to it and it was observed that catalase activity of stomach tissue increases significantly than control.

Keywords

Motility, Intestine, Cry, Chyme, Gastrointestinal Tract

Introduction

Cry proteins are specifically toxic to several insect orders like Lepidoptera, Coleoptera, Hymenoptera and Diptera, and also to nematodes. The Cry proteins comprise at least 50 subgroups with more than 200 members. Cry proteins are defined as: a parasporal inclusion protein from Bt that exhibits toxic effects to a target organism, or any protein that has obvious sequence similarity to a known Cry protein (Parker and Feil, 2005). A previous study explains Cry toxins exert their toxicity when activated at alkaline pH of the digestive tract of exposed larvae, and, because the

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somatic morphology of the digestive system in mammalian body does not allow the activation. However, the study demonstrated that Bt spore-crystals induced hematotoxicity, particularly to the erythroid ancestry. Bt spore-crystals caused hemolysis in cell lines of rat, mouse, sheep, horse, and human erythrocytes and suggested that the plasma membrane of vulnerable cells (erythrocytes in this case) may be the primary target for these toxins (Hofte and Whiteley, 1989).

In vitro evaluation system for gastrointestinal motility through Dale's assessment of small digestive tract, following 3 months treatment with Bt Cry protein, shows more evasion in stomach related improvement in respected animal when stood out from control. Histological segment of indicated tissue like small digestive tract, internal organ and stomach was regularly stained with haematoxylin-eosin (H/E) staining and noticed microscopically.Bt Cry protein doesn't influence the stomach tissue or doesn't make any harm it and it was seen that catalase action of stomach tissue increments fundamentally than control. This research aimed to identify the histological differences in gastrointestinal system between the Bt Cry proteins treated and control male albino rats.

Methods and Materials

Hematoxylene Eosin Staining :Hematoxylin and eosin (H&E) are the principal stains applied for the demonstration of the nucleus and the cytoplasmic inclusions. Harri's hematoxylin (primary stain) contains alum and alum acts as a mordant that stains the nucleus light blue which turns red in the presence of acid. The cell difference is carried out by treating the tissue with an acid solution. Eosin is the counter (secondary) stain that imparts pink color to the cytoplasm and extracellular matrix.

Dales Experiment: Rodent forfeited and gathered the small digestive tract. Next the small digestive tract cut into little pieces and put away with Dale's fluid. Hook fixed toward one side of bubbler in Dale's shower. One end of the digestive system fixed to a snare of bubbler and one more with the lever.The shower containing Dale's liquid and air is risen through it consistently. After that temperature keep up with about 37°C. And then, at that point, record the development of the gut (Hukuhara and Fukuda, 1965)

Biochemical tests: SGOT & SGPT Tests from Stomach tissue: Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to □ -ketoglutarate to yield oxaloacetate and L-glutamate. Malate dehydrogenase (MDH) catalyzes the reduction of oxaloacetate with simultaneous oxidation of NADH+ to NAD. The resulting rate of decrease in absorbance at 340 nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum. Alanine aminotransferase (ALT) catalyzes the transfer of the amino group from L-alanine to □ -ketoglutarate resulting in the formation of pyruvate and Lglutamate. Lactate dehydrogenase. LDH catalyzes the reduction of pyruvate and the simultaneous oxidation of NADH+ to NAD. The resulting rate of decrease in absorbance at 340 nm is directly proportional to ALT activity.

Catalase Test: 20 micro lit tissue homogenate taken in a test tube and 2 ml of H_2O_2 added

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carefully without any type of shaking. Then 0.5ml of PBS added in the test tube and the OD value was measured at 240nm.

Results and Discussion

In vitro assessment procedure for gastrointestinal motility through study by Dale's analysis of small digestive system, confined from both control and treated rodents following 3 months taking care of Bt Cry protein, shows more redirection in digestive development in regarded creature when contrasted with control . T3 test gathering of treated rodents is more noticeable among them. Various examples of digestive developments are associated with the physiological movement of chyme along the gastrointestinal lot (GIT) and are the aftereffect of the exchange between unconstrained action of digestive smooth muscle, intestinal or inherent and outward neural circuits. Here likewise unique example noticed. The investigation of gastrointestinal motility might be useful in deciding change in motility, assessing impact of neurotic condition on GI travel and helps in deciding the remedial capability of medications in motility problems. Histological segment of determined tissue like small digestive tract, internal organ and stomach was regularly stained with haematoxylin-eosin (H/E) staining and noticed infinitesimally.

Both AST or SGOT and ALT or SGPT tests is utilized to test organ harm because of medication, illness or injury. In case of liver tissue, heart tissue both are estimated and their expanded worth in blood demonstrate liver harm or coronary episode or cardiovascular breakdown. Here in the event of stomach tissue of both control and treated rodents SGOT esteem was diminished for T1 and T2 test gathering of treated rodents though expanded for T3, T4 and fundamentally for T5, in this manner it can't finish up particularly. Then again, the fig:11 showed a diminished degree of SGPT esteem when contrasted with control in this manner it tends to be presumed that Bt Cry protein doesn't influence the stomach tissue or doesn't make any harm it.

Further studies are required to emphasize on the motility of the small intestine and the cystic appearances observed after sacrificing of the albino rats. The results of the analysis of intestinal movements are shown in Figure 1. The results of kymograph recording of intestinal movements are shown in Figure 2, 3, and 5.

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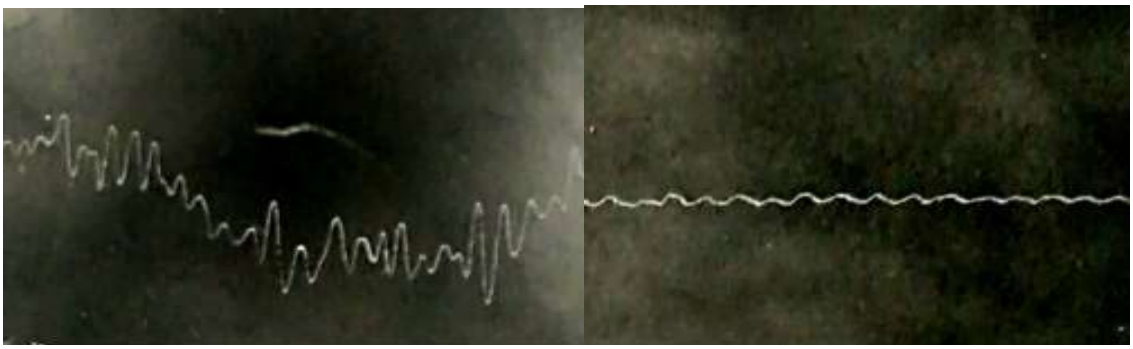

Figure 1. Visualizing the recordings from colon part. Contractions frequency and amplitude increased after administration of cry protein in diet.

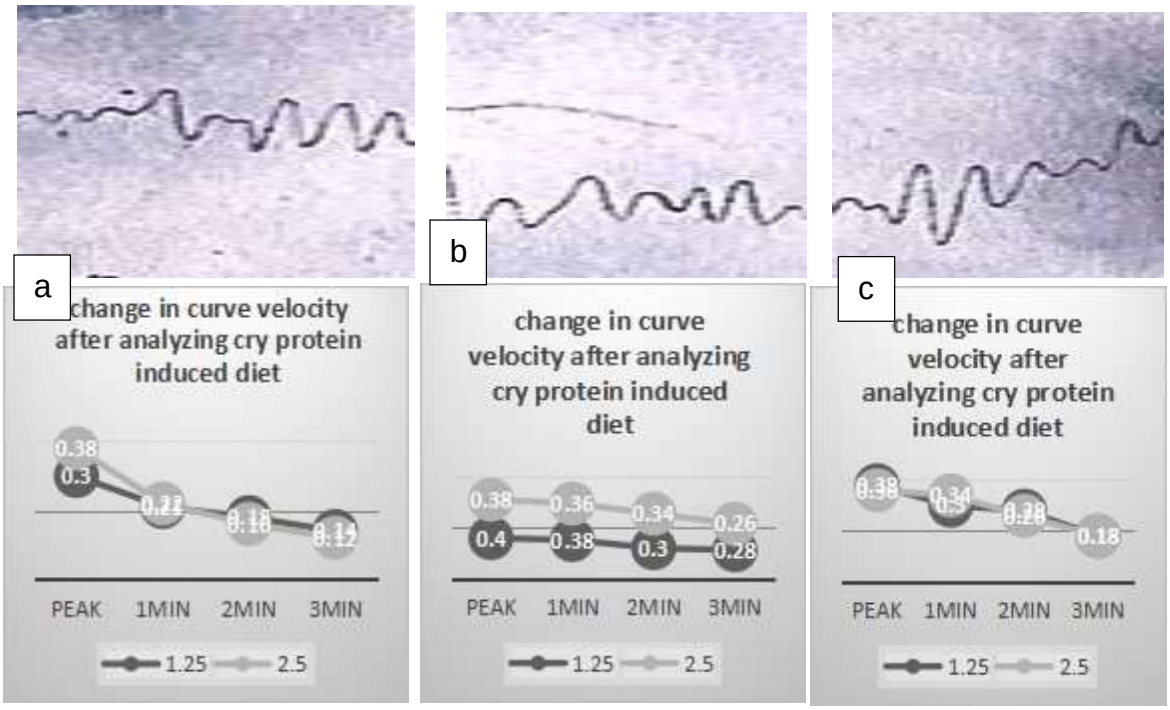

Figure 2. Different parts of colon showed different amplitudes varying with drum speed changes with the change in time. (a) Frontal part, (b) intermediate part and (c) peripheral part.

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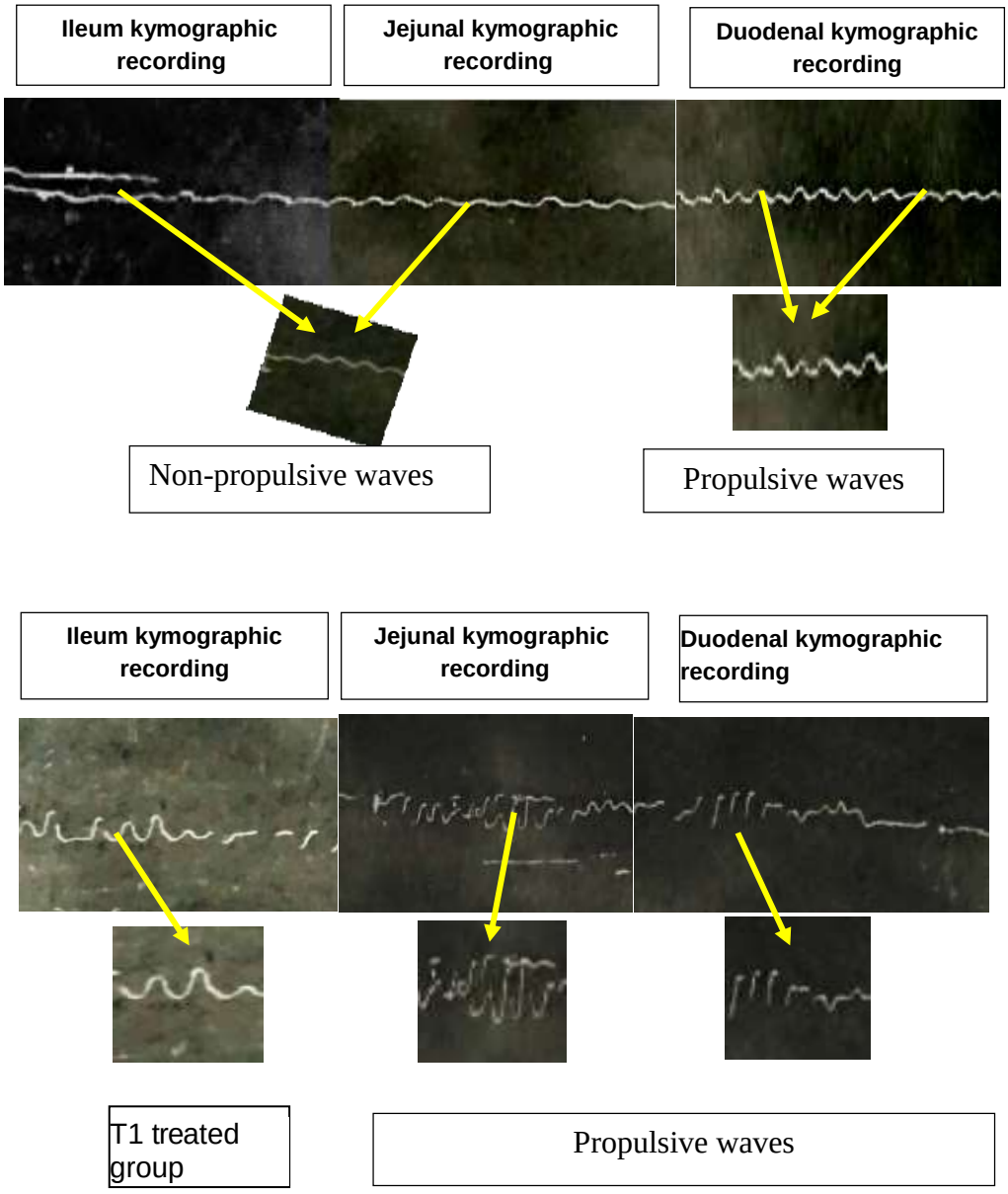

Figure 3. Kymographic recordings showing the propulsive and non- propulsive waves occurring during the experimental recordings of different parts of small intestine in Dales apparatus. The duodenal recording differs from the jejunal an ileum recording of the small intestine incase of thee propulsion reflex in case of normal recordings.

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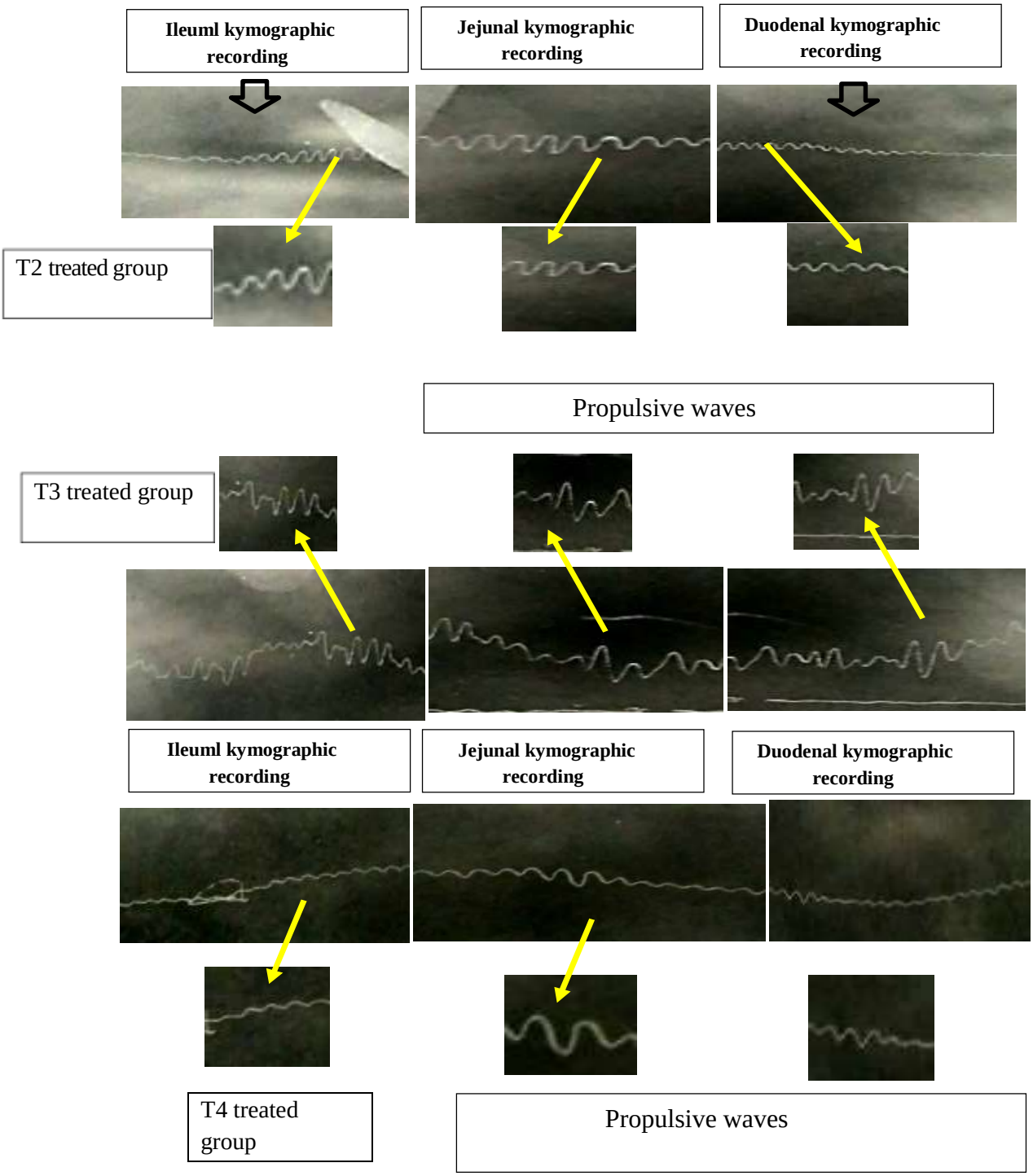

Figure 4. All the intestinal segments including duodenum, jejunum and ileum showed propulsive reflexes during the kymographic recording in case of the group of Cry protein treated rats.

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Cry toxins bind to the cellular brush border membrane vesicle (BBMV) of mammalian intestinal cells,it was found that Cry toxin did bind to the bovine and porcine BBMV, but far more weakly. The results of histological and histopathological changes are shown in Figure 5, 6, 7, and 8.

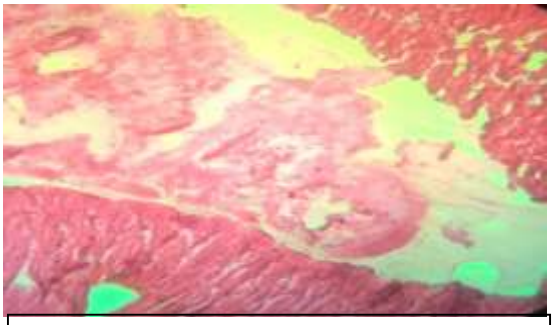

Figure 5. Histology of large intestine of the control rat.

Magnification 100X

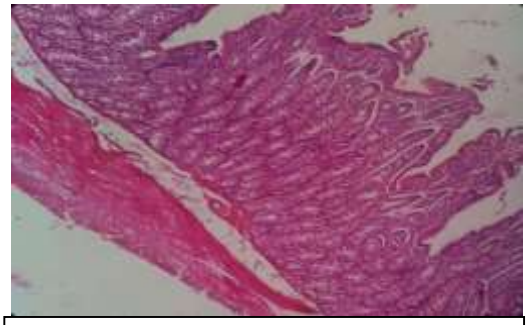

Figure 6. Denotes the treated rat's histology after feeding cry protein which shows degenerated cell membranes of the respective intestinal lineage cells along with degenerated and lobular appearance.

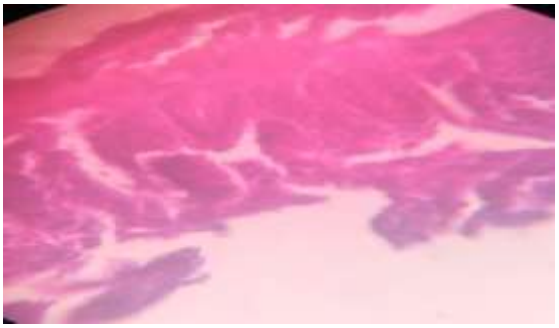

Figure 7. Histology of small intestine of the control rat.

Magnification 100X

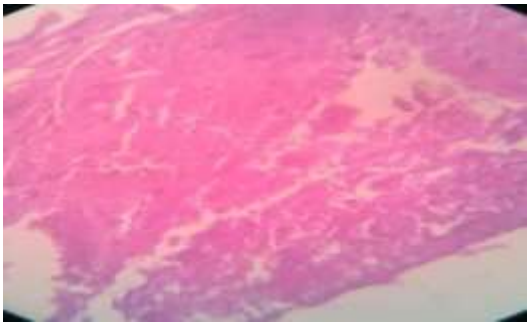

Figure 8. Denotes the treated rat's histology after feeding of cry protein which shows degenerated cell membranes of the respective intestinal lineage cells and teared appearance

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The experiments on the effects on SGOT and SGPT concentration of stomach, and catalase concentration, yield the results as shown in Figure 9, 10, and 11.

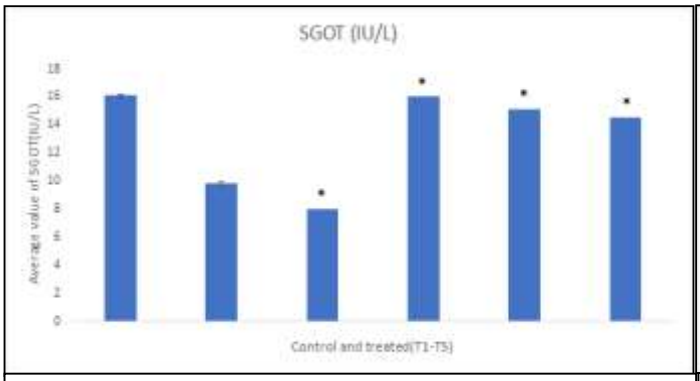

Figure 9:Diagram showing the difference between treated and untreated albino rat in case of SGOT test(IU/L)Se value=0.100047

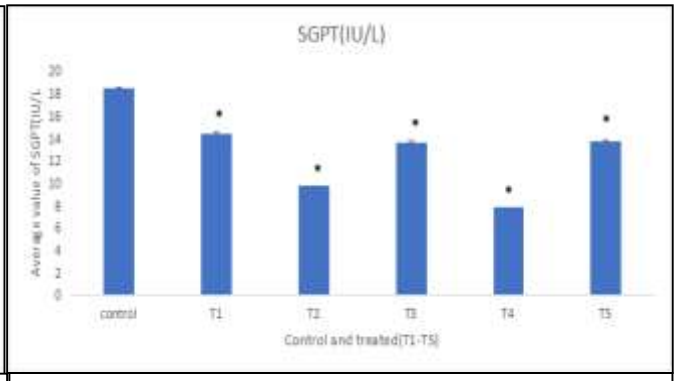

Figure 10:Diagram showing the difference between treated and untreated albino rat in case of SGPT test(IU/L)Se value=0.150918

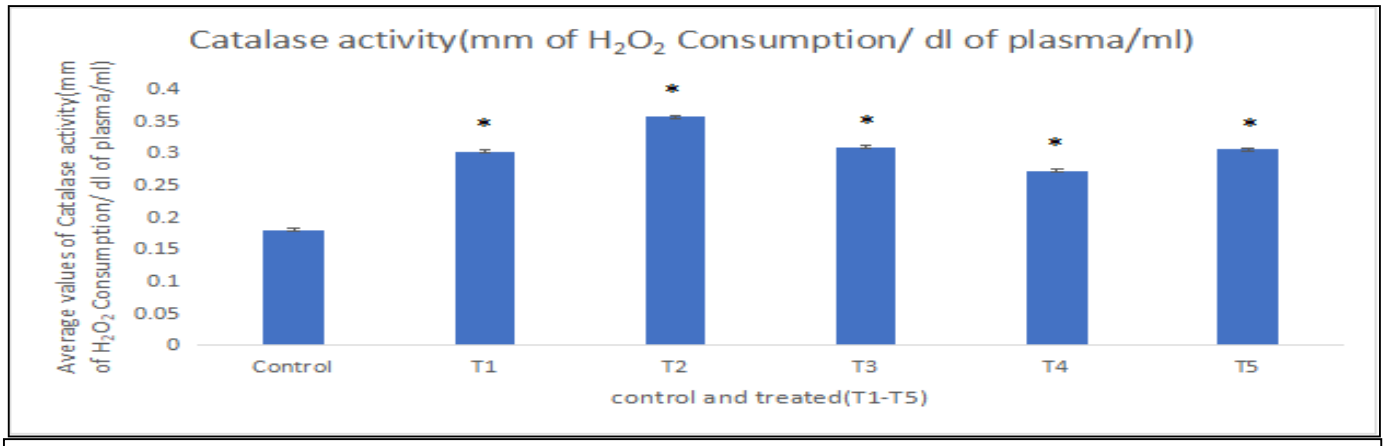

Figure 11: Diagram showing the difference between treated and untreated albino rat in case of Catalase activity of stomach tissue (mm of H2O2 Consumption/ dl of plasma/ml)

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